Proposed Registration Decision PRD2010-16

Pseudomonas syringae strain ESC-10

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Overview

Proposed Registration Decision for *Pseudomonas syringae* – strain ESC-10.

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Pseudomonas syringae* – strain ESC-10 Fungicide Technical and Bio-save 10LP Biological Fungicide, containing the technical grade active ingredient *Pseudomonas syringae* – strain ESC-10 to prevent fungal rot in stored fruits (apples, cherries and pears) and potatoes.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of *Pseudomonas syringae* – strain ESC-10 Fungicide Technical and Bio-save 10LP Biological Fungicide.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (e.g. children) as well as organisms in the environment (e.g. those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the PMRA's website at healthcanada.gc.ca/pmra.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act.*

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (*a*) efficacy; (*b*) effect on host organisms in connection with which it is intended to be used; and (*c*) health, safety and environmental benefits and social and economic impact."

Before making a final registration decision on *Pseudomonas syringae* – strain ESC-10, the PMRA will consider all comments received from the public in response to this consultation document[3]. The PMRA will then publish a Registration Decision[4] on *Pseudomonas syringae* – strain ESC-10 which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What Is *Pseudomonas syringae* – strain ESC-10

Pseudomonas syringae strain ESC-10 is a non-genetically engineered bacteria isolated from apples which is used as a microbial pest control agent (MPCA). This organism enters injuries on fruit or tubers where disease causing fungal spores are present and it then out-competes the fungal spores for nutrients. *Pseudomonas syringae* strain ESC-10 suppresses postharvest diseases on produce in storage facilities and packing houses. These diseases include blue mould and grey mould on apples, pears and cherries, mucor rot on apple and pear and dry rot on potato.

Health Considerations

Can Approved Uses of *Pseudomonas syringae* strain ESC-10 Affect Human Health?

***Pseudomonas syringae* strain ESC-10 is unlikely to affect your health when Bio-Save 10LP Biological Fungicide is used according to the label directions.**

People can be exposed to *P. syringae* strain ESC-10 when handling and applying the product and when consuming treated produce. When assessing health risks, several key factors are considered: the microorganism's biological properties (e.g., production of toxic byproducts); reports of any adverse incidents; its potential to cause disease or toxicity as determined in toxicological studies and the levels to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism. When *P. syringae* strain ESC-10 was tested on laboratory animals, there were no signs that it caused any toxicity or disease.

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3 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

4 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

Residues in Water and Food

Dietary risks from food and water are not of concern

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the Pest Control Products Act (PCPA) for the purposes of the adulteration provision of the Food and Drugs Act (FDA). Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

Strains of *P. syringae* are common in nature. The residues of *P. syringae* strain ESC-10 remaining on treated produce are expected to be higher than levels that naturally occur. When *P. syringae* strain ESC-10 was administered orally to rats, no signs that it caused toxicity or disease were observed and no metabolites of toxicological significance have been shown to be produced by this or other strains of *P. syringae*. Therefore the establishment of a MRL is not required for *P. syringae* strain ESC-10. As well, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently, dietary risks are minimal to non-existent.

Occupational Risks From Handling Bio-Save 10LP Biological Fungicide

Occupational risks are not of concern when Bio-Save 10LP Biological Fungicide is used according to label directions, which include protective measures

Workers using Bio-Save 10LP Biological Fungicide can come into direct contact with *P. syringae* strain ESC-10 on the skin, in the eyes, or by inhalation. For this reason, the label will specify that users exposed to Bio-Save 10LP Biological Fungicide must wear waterproof gloves, eye protection, long-sleeved shirts, long pants, and shoes plus socks and a dust/mist filtering NIOSH approved respirator/mask (with any N, P, R or HE filter).

For bystanders, exposure is expected to be much less than that of workers involved in loading and application activities and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When *Pseudomonas syringae* strain ESC-10 Is Introduced Into the Environment?

Environmental risks are not of concern

Pseudomonas syringae strain ESC-10 is a natural strain of bacteria that occurs on many kinds of plants throughout the world. Testing for pathogenicity on plants has shown that *Pseudomonas syringae* strain ESC-10 can be pathogenic to plants. However, since the use of Bio-Save 10LP Biological Fungicide is limited to enclosed spaces, exposure to non-target organisms, including plants, is negligible. Therefore the environmental risks are very low.

Value Considerations

What Is the Value of Bio-Save 10LP Biological Fungicide?

Bio-Save 10LP Biological Fungicide is a reduced risk bio-fungicide that suppresses diseases on produce in storage.

An increase in resistance of pathogens to commonly used chemical fungicides has been observed in some postharvest treatments for fruit crops. Resistance to the active ingredient, *Pseudomonas syringae* strain ESC-10, is unlikely to develop. This product has the potential to become an integral part of a postharvest IPM program.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the label of Bio-Save 10LP Biological Fungicide to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

Because of concerns with users developing allergic reactions through repeated high exposures to *P. syringae* strain ESC-10, anyone handling, mixing/loading, or involved in clean-up/repair activities of Bio-Save 10LP Biological Fungicide must wear waterproof gloves, a long-sleeved shirt, long pants and a dust/mist filtering respirator/mask (MSH/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator with any N-95, R-95, P-95 or HE filter. Eye protection is also required during loading activities.

Environment

As a general precaution, statements will be added to the label to prohibit handlers from contaminating aquatic habitats including lakes, streams, ponds or other waters.

Next Steps

Before making a final registration decision on *Pseudomonas syringae* strain ESC-10, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Pseudomonas syringae* strain ESC-10 (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Pseudomonas syringae strain ESC-10

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Pseudomonas syringae* strain ESC-10
Function	Suppress diseases on produce in storage
Binomial name	*Pseudomonas syringae* strain ESC-10
Taxonomic designation	
Kingdom	Prokaryotes
Phylum	Gracilicutes
Class	Scotobacteria
Order	Pseudomonodales
Family	Pseudomonoadaceae
Genus	*Pseudomonas*
Species	*Syringae*
Strain	ESC-10
Patent Status information	No patents are held by the applicant in Canada.
Minimum purity of active	1.0×10^{12} colony forming units (CFU)/g
Identity of relevant impurities of toxicological, environmental and/or significance.	The TGAI does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards. *Pseudomonas syringae* strain ESC-10 does not produce any known toxins or any other known toxic metabolites.

1.2 Physical and Chemical Properties of the Technical Grade Active Ingredient and the End-use Product

Technical Grade Active Ingredient – *Pseudomonas syringae* Strain ESC-10

Physical state	Paste
pH	6.5
Guarantee	minimum of 1.0×10^{12} colony forming units (CFU)/g

End-Use – Bio-Save 10 LP Biological Fungicide

Physical state	Aqueous suspension
Guarantee	1.0×10^{7} CFU/mL (nominal)
Colour	Clear
Odour	Odourless
pH	7.0
Density	1 kg/L (bulk)

1.3 Directions for Use

A single application of Bio-Save 10LP Biological Fungicide is applied to fruit or tubers as a dip or drench treatment or with an overhead application system. The product is intended as a post harvest application only and should be applied prior to disease development.

On cherries, mix 500 g of Bio-Save 10LP with 100 L water and apply 4 L of the solution to 900 to 1800 kg of cherries.

On apples and pears, apply a solution of 500 g Bio-Save 10LP in 300 L water to freshly cleaned fruit prior to waxing.

On potatoes, mix 500 g of Bio-Save 10LP with 100 L water and apply the entire suspension to 3000 sacks (45.5 kg each) of potatoes.

1.4 Mode of Action

The mode of action is competitive exclusion. The organism enters injuries (wounds, bruises) on fruit or tubers where fungal spores occur and it out-competes the spores for nutrients. This interrupts normal metabolism of the disease organism and does not allow the organism to grow or multiply.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

The active ingredient is identified to the genus level by a series of tests used to distinguish it from other plant pathogenic bacteria. Phenotypic tests are capable of identifying the microbial pest control agent (MPCA) as a unique *Pseudomonas syringae* strain. Fatty acid methyl ester (FAME) analysis matches the MPCA to a number of *P. syringae* pathovars (similarity indices of 0.876-0.943) and restriction fragment length polymorphism (RFLP) analysis followed by Southern blot analysis yields a unique banding pattern for *P. syringae* strain ESC-10 as compared to a number of pathovars of *P. syringae*, including two of the pathovars matched in FAME analsyis.

2.2 Methods for Establishment of Purity of Seed Stock

A cell bank of *P. syringae* strain ESC-10 is stored in liquid nitrogen and is deposited in the American Type Culture Collection (ATCC) in sufficient quantities to last many years of production. A master stock culture is prepared from the cell bank and is, in turn, used to generate the working stock. The master and working stock cultures are subjected to all standard quality control tests as well as streaking on agar to examine the cultures for purity. Bioassay, cellular fatty acid testing and rRNA sequence testing are periodically performed to ensure genetic stability and purity.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The guarantee of the end use product is based on the number of colony forming units per gram of product. Dilutions of the product are spread onto the surface of trypticase soy agar plates and the resulting colonies are counted in order to calculate the viable cell count.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

Although certain plant-pathogenic pathovars of *P. syringae* produce chlorosis-inducing toxins and/or necrosis-inducing toxins, *P. syringae* strain ESC-10 does not produce any known toxic substances. The mode of action of the MPCA is not toxin-mediated but is rather believed to be competition with mould- and rot-causing fungi for space and nutrients. Furthermore, the results of supporting mammalian toxicity and pathogenicity data do not indicate any toxic or pathogenic effects.

Based on the above information, the establishment of a maximum residue limit (MRL) is not required for *P. syringae* strain ESC-10 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulations. As a result, no methods to determine and quantify the MPCA and relevant metabolites are required.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality control procedures used to limit contaminating microorganisms during manufacture of *Pseudomonas syringae* Strain ESC-10 and Bio-Save 10 LP Biological Fungicide are acceptable.

Any product that does not meet the applicant's specifications for microbial contamination is destroyed.

2.6 Methods to Show Absence of Any Human and Mammalian Pathogens

As noted in section 2.5, quality control procedures are used to limit microbial contamination in *Pseudomonas syringae* Strain ESC-10 Fungicide Technical and Bio-Save 10 LP Biological Fungicide. These procedures include contamination checks to detect contaminating microbes.

Acceptable microbial contaminant analysis data were submitted for five batches of Bio-Save 10 LP Biological Fungicide.

2.7 Methods to Determine Storage Stability, Shelf-life of the Microorganism

The viability of *P. syringae* strain ESC-10 in Bio-Save 10 LP Biological Fungicide was evaluated over a 12 month period at 4°C and at 23°C. The submitted storage stability data support a storage period for Bio-Save 10 LP Biological Fungicide of up to one year at 4°C and for up to three months at 23 °C.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

The PMRA conducted a detailed review of the toxicological database for *P. syringae* strain ESC-10. The database for *Pseudomonas syringae* strain ESC-10 Fungicide Technical is complete (see Table 1), consisting of laboratory animal (in vivo) toxicity studies (acute oral toxicity/pathogenicity, acute pulmonary toxicity/pathogenicity and acute intravenous infectivity) currently required for health hazard assessment purposes. These studies were carried out in accordance with currently accepted international testing protocols and good laboratory practices. In addition to the studies on the technical product, acute oral and dermal toxicity /irritation and eye irritation studies were submitted for a 10 % by weight (*P. syringae* strain ESC-10) end-use formulation. Waiver requests were deemed acceptable to address the genotoxic and hypersensitivity potential of *P. syringae* strain ESC-10. The scientific quality of the data is high and the database is considered sufficient to characterize the toxicity and infectivity of this pest control agent and the end use product.

In an acute oral toxicity/pathogenicity study, fasted ~9 week old Sprague-Dawley rats (15/sex) were given by gavage, a single 1 mL dose of *P. syringae* strain ESC-10 at a concentration of 2.8×10^7 CFU/mL per animal. Four additional animals per sex (2 room controls and 2 shelf controls) were untreated and served as controls. After a 22 day observation period, there were no treatment related mortalities, clinical signs, necropsy findings or changes in body weight. The MPCA was detected in the cecum of a female rat on Day 22, but was not reported in the digestive tract of any other treated animal by Day 8. This study was classified as acceptable and based on the results there was no evidence of pathogenicity related to treatment with *P. syringae* strain ESC-10 at 2.8×10^7 CFU per animal via the oral route.

In an acute oral toxicity study, fasted young adult albino Sprague-Dawley rats (5/sex) were given a single oral dose of the test substance, an end-use formulation containing *P. syringae* strain ESC10 (10% by weight) at a dose level of 5010 mg/kg bw (at 1.46×10^{10} CFU/g). The animals were observed for a period of up to 14 days. No mortality, treatment related clinical signs, necropsy findings or significant changes in body weight were reported. This study was classified as acceptable and based on the result, there was no evidence of pathogenicity related to treatment with *P. syringae* strain ESC-10 at a dose level of 7.3×10^9 CFU /kg bw via the oral route. The LD_{50} for both male and female rats is greater than 5.0 g/kg of body weight of this end-use formulation containing *P. syringae* strain ESC-10 (10% by weight).

Pseudomonas syringae strain ESC-10 when administered by the intratracheal route at a dose of 8.4×10^8 CFU per animal, did not cause any signs of pathogenicity or infectivity in the rat. In an acute pulmonary infectivity and toxicity study 8 week old Sprague-Dawley rats, 57 animals per sex were exposed by the intratracheal route to *P. syringae* strain ESC-10 in sterile phosphate buffered saline (2.1×10^{10} CFU/mL) at a single dose of 8.4×10^8 CFU/animal. An additional group of 6 animals per sex was used as an untreated control. Animals were observed for up to 22 days and at necropsy, no gross lesions or other evidence of pathogenicity was reported in any of the test animals. The MPCA was detected in the lungs of all treated animals on Day 1 of the study but by Day 4, and at all subsequent sacrifice days, there were no detectable levels in lung homogenates suggesting complete clearance from the lungs.

The MPCA was not detected in any other tissues or organs of the treated animals at any time point, except for one female rat in which a small number of colonies in the mesenteric lymph nodes was detected on Day 8 of the study. This infectivity and toxicity testing of *P. syringae* strain ESC-10 in male and female Sprague-Dawley rats was classified as acceptable. Based on these results there was no evidence of treatment related toxicity or infectivity to a single dose of 8.4×10^8 CFU/animal of *P. syringae* strain ESC-10.

In an acute intravenous infectivity study, 8 week old Sprague-Dawley rats (18 animals per sex) were injected in the tail vein with the MPCA (*P. syringae* strain ESC-10) in sterile phosphate buffered saline, at a dose of approximately 10^7 CFU/animal. An additional group of 5 animals per sex was used as an untreated control. Animals were then observed for up to 22 days. With the exception of Day 15 females, body weight gain was not significantly affected in the treated groups when compared statistically with the control values. No clinical signs of toxicity or gross lesions were observed in any of the test animals and there was no evidence of pathogenicity. Microbial counts were reported in the blood of 5 of the 6 treated animals on Day 1 but were no

longer detected by Day 4. Low and sporadic microbial counts were reported in the spleen on Day 4, but were no longer detected in any tissue after Day 8. This pathogenicity testing of *P. syringae* strain ESC-10 in male and female Sprague-Dawley rats was classified as acceptable. Based on the results of this study, there was no pathogenicity related to treatment by intravenous injection with *P. syringae* strain ESC-10 at a dose of approximately 10^7 CFU/animal.

In an acute dermal toxicity study, 10 New Zealand rabbits (six males and four females) were dermally exposed to the test article, an end-use formulation containing *P. syringae* strain ESC-10 (10% by weight) at a dose of at least 5010 mg/kg (at 1.46×10^{10} CFU/g), applied to an area of approximately 10% of the body surface and then covered for 24 hours. Following exposure, the animals were observed for a period of 15 days. No adverse effects, or signs of toxicity were reported and all test rabbits gained weight during the study. This toxicity testing of *P. syringae* strain ESC-10 in male and female New Zealand rabbits was classified as acceptable. Based on the results of this study there was no systemic or local toxicity related to the dermal application of the test article, containing *P. syringae* strain ESC-10 (10% by weight) at a dose of at least 5010 mg/kg (at 1.46×10^{10} CFU/g).

In a primary dermal irritation study 6 male New Zealand White rabbits were dermally exposed to the test substance, an end-use formulation containing *P. syringae* strain ESC-10 (10% by weight) at a dose of 500 mg (1.46×10^{10} CFU/g) for 4 hours. Animals then were observed for 3 days. Irritation was scored by the method of Draize scale for scoring primary skin irritation. Very slight grade erythema (Grade 1) was observed in two of six rabbits 30 to 60 minutes and 24 hours after unwrapping. Irritation was scored by the Draize method and the calculated maximum irritation score (MIS) after 24 hours was 0.333/4 with a mean average score (MAS) of 0.111/4. The erythema was resolved by the 48 hour observation point, and no other indications of a dermal effect were reported. This primary dermal irritation study was classified as acceptable. Based on the results of this study the test substance containing *P. syringae* strain ESC-10 (10% by weight) was determined to be minimally irritating to the skin of New Zealand rabbits at a dose of 500 mg (at 1.46×10^{10} CFU/g).

In a primary eye irritation study, 100 mg of the test substance, an end use formulation containing *P. syringae* strain ESC-10 (10% by weight) was instilled into the conjunctival sac of one eye each of six New Zealand White rabbits for 7 days with no subsequent wash out. The treated eye was examined 1, 24, 48 and 72 hours and 4 and 7 days after treatment for signs of irritation. Animals then were observed for 7 days. Irritation was scored by the method of Draize and the calculated maximum irritation score (MIS) after 24 hours was 4.33/110 with a mean average score (MAS) of 1.11/110. Minimal irritation was reported in all six rabbits at 1 hour and in 4 of 6 rabbits at 24 hours. The irritation was resolved by 48 hours in 5 of 6 rabbits and in the remaining rabbit before the Day 5 observation. This study was classified as acceptable and under study conditions, ocular application of 100 mg of the test article, resulted in minimal, reversible eye irritation.

It should be noted that the toxicity testing carried out with the end-use formulation contained the MPCA at 10% by weight, whereas the end use formulation being proposed for registration contains *P. syringae* strain ESC-10 Technical specified at 29.8% by weight. Based on the lack of adverse effects in testing conducted with the lower MPCA concentration, and lack of toxicity expected from the formulation ingredients of Bio-Save 10LP Biological Fungicide, no further testing with the higher concentration of the MPCA formulated product is required.

The applicant indicated that no hypersensitivity incidents had been reported for researchers or workers handling the product during studies to support the application for registration of the product Bio-Save 10LP Biological Fungicide. Furthermore, no reports of hypersensitivity to these bacteria have been reported in the published literature. Nevertheless, because most microorganisms contain substances that elicit positive hypersensitivity reactions in humans, *P. syringae* strain ESC-10 is considered to be a potential sensitizing agent. Consequently the signals words "POTENTIAL SENSITIZER" are required on the principal display panels of the technical product *P. syringae* strain ESC-10 Fungicide Technical and the end use product Bio-Save 10LP Biological Fungicide labels.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the MPCA, and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral, intravenous and pulmonary toxicity/infectivity tests.

Within the available scientific literature, there are no reports that suggest *P. syringae* strain ESC-10 has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral, intravenous and pulmonary routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated from exposure to *P. syringae* strain ESC-10.

3.2 Occupational/Bystander Exposure and Risk Assessment

3.2.1 Occupational

Bio-Save 10LP Biological Fungicide is a post-harvest treatment being proposed for use in produce processing and packing facilities to apples, cherries, pears and potatoes intended for long term storage. Application to cleaned produce is being proposed by dip/drench techniques or overhead sprays. When handled according to the proposed label instructions, the potential routes of worker exposure to Bio-Save 10LP Biological Fungicide containing the active ingredient *P. syringae* strain ESC-10 are dermal, pulmonary and to some extent ocular. However, the PMRA does not expect that the occupational exposures from the proposed uses in produce processing and packing facilities will be of concern on the basis of the low toxicity/pathogenicity profile for *P. syringae* strain ESC-10 and associated end-use formulation, and on the assumption that the precautionary labelling instructions aimed at minimizing worker exposure are adhered to by users.

Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. This MPCA has not been identified as a wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Although no dermal toxicity and little dermal irritation are expected based on toxicological studies of the MPCA and toxicological characteristics of the formulation ingredients present in the end-use formulation, all MPCAs are considered potential sensitizers. The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions. Label restrictions and risk mitigation measures are required to protect populations that are likely to be primarily exposed to the products. Such exposure to loaders, handlers and other workers can be minimized if they wear water proof gloves, long-sleeved shirts, long pants, shoes and socks.

Overhead spraying of the MPCA to agricultural produce on a conveyor system has the potential to result in inhalation exposure to workers. However, the use pattern is such that spray mists are contained, recovered and reused, thus reducing the likelihood for worker exposure during overhead spray operations. Workers loading the wettable powder formulation for dip/drench or spray applications are at risk for inhalation of dusts and/or particulate matter. Based on the toxicological profile for *P. syringae* strain ESC-10, exposure to a large single quantity of the MPCA via the pulmonary route is not of concern. However, respiratory hypersensitivity could possibly develop upon repeated exposure to the product. Exposure in workers will be mitigated by a label requirement for persons loading Bio-Save 10LP Biological Fungicide to wear personal protective equipment, including a dust/mist filtering respirator/mask (MSH/NIOSH approval number prefix TC-21) or NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products.

Based on the results of the eye irritancy study, Bio-Save 10LP Biological Fungicide is expected to cause minimal eye irritation on exposure, with the effects being reversible. The end-use formulation being proposed for registration is a wettable powder and the potential for ocular exposure is greatest during loading activities. Exposure in workers will be mitigated by a label requirement for eye protection to be worn by persons loading the wettable powder formulation, in addition to the required personal protective equipment (PPE).

3.2.2 Bystander

Inhalation or dermal exposure to the general public is expected to be low from the proposed indoor dip/drench and spray applications of Bio-Save 10LP Biological Fungicide to raw agricultural commodities. Overall the PMRA does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for *Pseudomonas syringae* strain ESC-10 Technical Fungicide and its associated end-use formulation Bio-Save 10LP Biological Fungicide.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

The residues of *P. syringae* strain ESC-10 remaining on produce from the indoor use of Bio-Save 10LP Biological Fungicide are expected to be higher than levels naturally occurring on fruit. There is however, negligible to no risk expected for the general population, including infants and children, or animals because *P. syringae* strain ESC-10 demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity/infectivity rat study. No secondary metabolites of toxicological significance have been shown to be produced by this or other isolates of *P. syringae*.

Studies designed to determine the concentration of *P. syringae* strain ESC-10 remaining on pears after a spray application, and apples after dip treatment with Bio-Save 10LP Biological Fungicide were submitted for review. The studies included industry cultural practices such as drying of fruit at 60°C and cold storage. Although these non-guideline studies were classified as supplemental, results do confirm that *P. syringae* strain ESC-10 does remain on the fruits as residual matter after treatment. However there are no human health concerns based on the low toxicity of the MPCA and no indications of infectivity, toxicity or pathogenicity as demonstrated in the submitted animal studies.

Furthermore, higher tier subchronic and chronic dietary exposure studies were not required because of the low toxicity of the MPCA and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity studies. Therefore, there are no concerns for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.3.2 Drinking Water

The likelihood that *P. syringae* strain ESC-10 could enter neighbouring aquatic environments as a result of the proposed use indoors, in produce storage and processing facilities is negligible. No risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and because there were no harmful effects observed in Tier I acute oral toxicity and infectivity testing. The Bio-Save 10LP Biological Fungicide label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Users are also requested not to allow effluent or runoff from produce treatment/storage facilities containing this product to enter lakes, streams, ponds or other waters. Furthermore, municipal treatment of drinking water is expected to remove the transfer of residues to drinking water. Therefore, potential exposure to *P. syringae* strain ESC-10 in surface and drinking water is negligible.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARDs) and acceptable daily intakes (ADIs) are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e., no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that the MPCA is of low toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCA than the general population. Thus there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects from pre- or post-natal exposures, and cumulative effects of the MPCA and other registered micro-organisms that have a common mechanism of toxicity on infants and children, does not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of this MPCA to human health.

3.4 Maximum Residue Limits

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the Pest Control Products Act (PCPA) for the purposes of the adulteration provision of the Food and Drugs Act (FDA). Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

The post-harvest application of Bio-Save 10LP Biological Fungicide to agricultural produce intended for long term storage is expected to result in residues of *P. syringae* strain ESC-10 remaining on treated produce that are higher than levels naturally occurring on fruit. However, no adverse effects from dietary exposure have been attributed to natural populations of *P. syringae*, and no adverse effects were observed in the acute oral toxicity study with *P. syringae* strain ESC-10. Therefore the establishment of an MRL is not required for *P. syringae* strain ESC-10.

3.5 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA's files, there is reasonable certainty no harm will result from aggregate exposure of residues of *P. syringae* strain ESC-10 to the general Canadian population, including infants and children, when the microbial pest control product Bio-save 10LP Biological Fungicide is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information.

Furthermore, there have been no adverse effects from exposure to natural populations of *P. syringae* in the environment. Even if there is an increase in exposure to this microorganism from the food uses of Bio-Save 10LP Biological Fungicide, there should not be any increase in potential human health risk.

3.6 Cumulative Effects

The PMRA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Besides naturally occurring strains of *P. syringae* in the environment, the PMRA is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity with this active ingredient. No cumulative effects are anticipated if the residues of *P. syringae* strain ESC-10 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Environmental fate testing is intended to demonstrate whether a microbial pest control agent (MPCA) is capable of surviving or replicating in the environment to which it is applied, and could provide an indication of which non-target organisms may be exposed to the MPCA as well as provide an indication of the extent of exposure. Environmental fate data (Tier II/III) are not normally required at Tier I, and are only triggered if significant toxicological effects in non-target organisms are noted in Tier I testing. Since toxicological effects were not noted, no fate data are required to complete the environmental risk assessment of *Pseudomonas syringae* strain ESC-10 Fungicide Technical when formulated as the end-use product Bio-Save 10LP Biological Fungicide.

4.2 Effects on Non-Target Species

4.2.1 Effects on Terrestrial Organisms

The pathogenicity of *Pseudomonas syringae* strains ESC-10 and ESC-11 on the cut, surface sterilized, dormant shoots of sweet cherry (cv. Schmidt) and pear (cv. Bartlett) was studied at measured concentrations of 1.0×10^8, 1.0×10^7 and 1.0×10^6 colony forming units (CFU)/mL in accordance with the United States Environmental Protection Agency Office of Prevention, Pesticides and Toxic Substances (U.S. EPA OPPTS) 885.4300. The negative control group was inoculated with water. Three positive control groups were conducted in the same manner as the test groups; strains PSS508, PSS34 and PSS23 were used. *Pseudomonas syringae* strain ESC-11 showed no pathogenicity in the shoots of sweet cherry. *Pseudomonas syringae* strain ESC-10 showed signs of mild infection in two of the replicates in the form of bacterial ooze in the shoots of sweet cherry. One of the positive controls, PSS34, exhibited heavy bacterial ooze and extended blackening of the shoot on the inoculated end. This study is classified as acceptable.

The pathogenicity of *Pseudomonas syringae* strain ESC-10 on the shoots and leaves of sweet orange (*Citrus sinensis*), grapefruit (*Citrus paradisi*), lime (*Citrus aurantifolia*), lemon (*Citrus limon*) and avocado (*Persea americana*) was studied at measured concentrations of 1×10^4 (leaves), 1×10^5 (leaves), 1×10^6 (shoots and leaves), 1×10^7 (leaves), and 1×10^8 (leaves) CFU/mL; and on the mature fruit of lemon, grapefruit, lime and orange at measured concentrations of 1×10^5, 1×10^6, 1×10^7, and 1×10^8 CFU/mL in accordance with the U.S. EPA OPPTS 885.4300. Another strain of *Pseudomonas syringae* of unknown pathogenicity was tested concurrently, *Pseudomonas syringae* strain ESC-11. As well, a positive control group was tested using *Pseudomonas syringae* strain 0485-10, and a negative control using water. Dark brown areas 1 – 2mm in diameter were observed on lime fruit after inoculation with ESC-10; lesions 15 – 19mm in diameter were observed on lime fruit inoculated with the positive control strain, 0485-10. This study is classified as acceptable.

The effect of *Pseudomonas syringae* strains ESC-10 and ESC-11 on the blossoms of cherry, pear, prune, and apricot; the green shoots of cherry, prune, apple, peach and apricot; and the trunks of apple and peach was studied at measured concentrations of between 1.0×10^7 and 1.0×10^8 CFU/mL in accordance with the U.S. EPA OPPTS 885.4300. The negative control group was inoculated with sterilized distilled water. *Pseudomonas syringae* strain PS 508 was used as a control. *Pseudomonas syringae* strains PSS 12, PSS 23, and PSS 40 were used as reference strains. It was determined that strain ESC-10 and ESC-11 can cause infection on cherry and pear blossoms and cankers on peach shoots. This study is classified as acceptable.

In addition to the above terrestrial plant studies, several scientific rationales were submitted to waive testing on birds, mammals, arthropods, non-arthropod invertebrates and microorganisms based on the biological and ecological properties of the MPCA, and the limited potential for exposure from the use of Bio-Save 10LP Biological Fungicide.

Bio-Save 10LP Biological Fungicide will be applied by diptank, spray line, or drenches. These applications are considered indoor uses and therefore non-target species would not be expected to come in contact with the product. The active ingredient, *Pseudomonas syringae* strain ESC-10, belongs to a bacterial species that has worldwide distribution. *Pseudomonas syringae* strain ESC-10 does not grow at temperatures above 32°C. Most avian and mammalian species maintain a body temperature range of 38°C to 42°C. Given these criteria, if any avian or mammalian species were to come in contact with this product, no harmful effects would be expected.

Further to the submitted studies and waiver rationales, the OECD *Consensus Document on Information Used in the Assessment of Environmental Applications Involving Pseudomonas* contains the following details relevant to the environmental risk assessment for terrestrial organisms:

> *Pseudomonas syringae* occurs naturally among the microflora that inhabit the leaf surface of plants that are typically found in temperate and Mediterranean climates. *Pseudomonas syringae* survives in association with the host plant and propagative material from the host plant. There is little evidence to suggest that these bacteria survive in soil. They may, however, survive in soil in association with residues of diseased plants, having some capacity to colonize root systems (both host and non-host plant). Stone or pome

fruit pathogens, such as *Pseudomonas syringae*, exist in lesions, cankers or tumours. Inocula is therefore available for dissemination under favourable environmental conditions. Most of the *Pseudomonas syringae* group appears to have the capacity to survive as epiphytes on protected parts of healthy leaves, in the buds of the host, and even on non-host plants.

No reports were found of *Pseudomonas syringae* occurring as an animal pathogen.

Pseudomonas syringae is principally an assemblage of foliar pathogens, although it occurs as both pathogenic and epiphytic (non-pathogenic) strains. The species has a broad range of potential plant hosts. Pathogenic strains can exhibit both pathogenic (i.e. disease-causing) and epiphytic behaviours on susceptible hosts. The initiation of infection results when a threshold level of bacteria is reached on the leaf surface; in the case of *Pseudomonas syringae* pv. *syringae* this is reported to be 10^4 CFU/g of tissue.

The association between rain and the onset of foliar blights caused by *Pseudomonas syringae* is well recognized. Rain appears to stimulate the differential growth of pathogenic *Pseudomonas syringae* isolates from the heterogeneous populations (pathogenic and non-pathogenic strains). Rain-triggered growth of *Pseudomonas syringae* results in the establishment of large pathogenic populations required for disease development.

There appears to be a distinctive set of symptoms associated with each causal agent. *Pseudomonas syringae* pv. *savasatoni* incites tumourous outgrowths on stems and leaves of oleander and olive under natural conditions. These symptoms have been found to be associated with the production of the auxin, indole acetic acid (IAA), in tissues infected with the bacterium. Furthermore, chlorosis, a common symptom when plants are infected by a number of pathogens belonging to the *Pseudomonas syringae* group, is indicative of production of a toxin. For example, halo blight of beans caused by *Pseudomonas syringae* pv. *phaselicola* is mediated by the toxin, phaseolotoxin.

The *Dictionary of Natural Products* lists the following toxins produced by various strains of *P. syringae*: 1H-Indole-3-carboxaldehyde, octicidin (phytotoxin), phaseolotoxin (phytotoxin), N-Phosphosulfamylornithine (phytotoxin), syringomycin (phytotoxin), syringostatin A (phytotoxin), syringostatin B (phytotoxin), syringotoxin B (phytotoxin), tagetitoxin (phytotoxin), coronafacic acid (induces chlorosis in plants), halotoxin (phytotoxin), tabtoxin (phytotoxin). Coronatine (phytotoxin) is also produced by certain strains of *Pseudomonas syringae*. It was determined that syringomycin production was stimulated by iron and suppressed by inorganic phosphate, that production occurred between 15 and 27°C, and that a slow growth rate of *Pseudomonas syringae* favours toxin production.

Based on all the available data, waiver rationales and information on the effects of *Pseudomonas syringae* strain ESC-10 to terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, arthropods, non-arthropod invertebrates, plants or to other non-target microorganisms from the indoor use of Bio-Save 10LP Biological Fungicide. Residual MPCA found on treated seed potato are not expected to be mobile in soil since *Pseudomonas syringae* is not known to survive in soil, therefore, the resulting exposure to flora and fauna is

expected to be negligible. As well, *Pseudomonas syringae* strain ESC-10 is not known to produce any toxicologically significant metabolites, other than phytotoxins, nor is it known to be pathogenic to organisms outside of the plant kingdom.

4.2.2 Effects on Aquatic Organisms

Several scientific rationales were submitted to waive testing on freshwater fish, freshwater aquatic invertebrates and other estuarine and marine animals based on the biological and ecological properties of the MPCA, and the limited potential for exposure from the use of Bio-Save 10LP Biological Fungicide.

The use pattern for the product, *Pseudomonas syringae* strain ESC-10, dictates application by diptank, spray line or drench. These applications are considered indoor uses and therefore non-target species would not be expected to come in contact with the product. As well, there are reports in the published scientific literature of *Pseudomonas syringae* strain ESC-10 having toxic effects on freshwater fish or freshwater aquatic invertebrates.

Further to the waiver rationales, the OECD *Consensus Document on Information Used in the Assessment of Environmental Applications Involving Pseudomonas* (see Section 4.2.1 above) contains details relevant to the environmental risk assessment for aquatic organisms.

Based on all the available data, waiver rationales and information on the effects of *Pseudomonas syringae* strain ESC-10 to aquatic organisms, there is reasonable certainty that no harm will be caused to freshwater fish, estuarine or marine fish, aquatic arthropods, aquatic plants, or aquatic non-arthropod invertebrates from the indoor use of Bio-Save 10LP Biological Fungicide. Residual MPCA found on treated seed potato are not expected to be mobile in soil since *Pseudomonas syringae* is not known to survive in soil, therefore, the resulting exposure to aquatic organisms is expected to be negligible. As well, *Pseudomonas syringae* strain ESC-10 is not known to produce any toxicologically significant metabolites, other than phytotoxins, nor is it known to be pathogenic to organisms outside of the plant kingdom.

5.0 Value

5.1 Effectiveness Against Pests

5.1.1 Acceptable Efficacy Claims

A total of 64 trials were submitted in support of the use claims; 53 on apple and pear and 11 on potato. Eleven trials were not reviewed for the following reasons: trial reports were incomplete; assessed crops are not grown in Canada or were not proposed on the submitted label; or the trial employed an application method not proposed on the submitted label.

The data demonstrates that Bio-Save 10LP Biological Fungicide will suppress postharvest diseases in storage including blue mould (*Penicillium expansum*), grey mould (*Botrytis cinerea*) and mucor rot (*Mucor piriformis*) on apple and pear and dry rot (*Fusarium sambucinum*) on potato. The claims for suppression of these diseases are supported at the proposed rates and timing of application. No efficacy data was submitted on cherry. Use claims for suppression of blue mould and grey mould on apples and pears can be extrapolated to cherries because the diseases are caused by the same pathogens and the disease cycles are the same as that observed on these crops. The proposed rate and timing of application on cherries are supported. No phytotoxicity was observed in any trial.

Data submitted on control of dry rot on potato demonstrated that Bio-Save 10LP Biological Fungicide will provide consistent suppression of this disease when applied to tubers at the proposed rate and timing. No phytotoxicity was observed.

5.2 Phytopathogenicity

Phytopathogenicity of *P. syringae* strain ESC-10 was assessed on fruit trees including cherry, pear, peach and apple to determine if the use of this organism could cause infection in fruit orchards in the vicinity of packing houses. Three trials were submitted; one was not reviewed because the crops assessed are not grown in Canada. Results showed that *P. syringae* ESC-10 is capable of causing infection and/or ooze formation on cherry. However, it is unlikely that *P. syringae* ESC-10 will incite disease because infection must occur in the spring. *P. syringae* strain ESC-10 will be used in the fall and the inoculum is to be killed before leaving the packing house. Once mixed, it has a very short shelf life and sanitation cleaners used in storage facilities kill the product on contact. Due to these conditions, it is unlikely that infection of adjacent cherry orchards will occur.

5.3 Compatibility with crop protection and management practices

Bio-Save 10LP Biological Fungicide is compatible with other post harvest handling procedures for apples, pears, cherries and potatoes. Bio-Save 10LP Biological Fungicide does not interfere with the activity of other treatments such as diphenylamine, calcium chloride or chlorine dioxide. Waxes can be applied after the Bio-Save 10LP Biological Fungicide application with no injury to the active *P. syringae* cultures.

5.4 Economics

No market analysis was done for *Pseudomonas syringae* Strain ESC-10.

5.5 Sustainability

5.5.1 Survey of Alternatives

The chemical fungicides listed in Table 3, Appendix I, are registered for control or suppression of diseases on the crops found on the Bio-Save 10LP label.

5.5.2 Compatibility with Current Management Practices Including Integrated Pest

Management

Bio-Save 10LP Biological Fungicide has a different mode of action than the commercial standard fungicides for postharvest treatment of apples, pears, cherries and potatoes. It is an alternative product to currently registered fungicides which will aid in the management of fungicide resistance. Bio-Save 10LP Biological Fungicide can be considered a component of an IPM strategy for the management of postharvest diseases on these crops.

5.5.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

Bio-Save 10LP Biological Fungicide is a bio-fungicide. Resistance to the active ingredient *P. syringae* strain ESC-10 is unlikely to develop.

5.5.4 Contribution to Risk Reduction and Sustainability

Bio-Save 10LP Biological Fungicide is a biological product with a low risk of pest resistance development. This product is compatible with other postharvest treatments for the labelled crops. The active ingredient is effective against pests that have developed resistance to thiabendazole and is an alternative to chemical treatments currently in use by storage facilities and packing houses.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The management of toxic substances is guided by the federal government's Toxic Substances Management Policy (TSMP), which puts forward a preventive and precautionary approach to deal with substances that enter the environment and could harm the environment or human health. The policy provides decision makers with direction and sets out a science-based management framework to ensure that federal programs are consistent with its objectives. One of the key management objectives is virtual elimination from the environment of toxic substances that result predominantly from human activity and that are persistent and bioaccumulative. These substances are referred to in the policy as Track 1 substances.

In its review, the PMRA took into account the federal Toxic Substances Management Policy and followed its Regulatory Directive DIR99-03, The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy. Substances associated with its use were also considered, including microcontaminants in the technical product, *Pseudomonas syringae* – strain ESC-10 Fungicide Technical, and formulants in the end-use product, Bio-Save 10 LP Biological Fungicide. The PMRA has reached the following conclusions:

Pseudomonas syringae – strain ESC-10 Fungicide Technical does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. There are also no formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track 1 criteria.

Therefore, the use of *Pseudomonas syringae* – strain ESC-10 Fungicide and Bio-Save 10 LP Biological Fungicide are not expected to result in the entry of Track 1 substances into the environment.

6.2 Formulants and Contaminants of Health or Environmental Concern

The technical grade of the active ingredient (TGAI), *Pseudomonas syringae* – strain ESC-10 Fungicide Technical, does not contain any contaminants of health or environmental concern identified in the Canada Gazette, Part II, Volume 139, Number 24, pages 2641–2643: List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.

The end-use product, Bio-Save 10 LP Biological Fungicide, does not contain any contaminants of health or environmental concern identified in the Canada Gazette, Part II, Volume 139, Number 24, pages 2641–2643: List of Pest Control Product Formulants of Health or Environmental Concern.

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for *Pseudomonas syringae* – strain ESC-10 Fungicide Technical and Bio-Save 10 LP Biological Fungicide were deemed to be adequate to assess their potential human health and environmental risks. The TGAI was characterized and the specifications were supported by the analyses of a sufficient number of batches. Storage stability data were sufficient to support a shelf life of one year at 4°C or up to three weeks at 23°C.

7.1 Human Health and Safety

The acute toxicity and infectivity studies submitted in support of *P. syringae* strain ESC-10 were determined to be sufficiently complete to permit a decision on registration for indoor uses. *Pseudomonas syringae* strain ESC-10 was of low toxicity in the rat when administered via oral, pulmonary and dermal routes and was not infective via the pulmonary and intravenous routes of exposure with a pattern of clearance established by Day 21.

The eye and dermal irritancy of *P. syringae* strain ESC-10 in a formulated product have been addressed in animal studies, following exposure to 10^8 CFU. Based on the results of the submitted studies, Bio-Save 10LP Biological Fungicide is expected to cause minimal irritation to the eye and skin on exposure, with the effects being reversible.

When handled according to the label instructions, the potential for dermal, eye and pulmonary exposure for persons loading the MPCA exists, with the primary source of exposure to workers being dermal and to a lesser extent inhalation. Precautionary statements on product labels and the wearing of personal protective equipment (PPE) including protective eye wear will adequately mitigate the risks from exposure.

While *P. syringae* strain ESC-10 has the potential to be a sensitizing agent, inhalation and dermal exposure is not a concern if the required dust/mist filtering respirator/mask and appropriate PPE stipulated on the end-use product label is worn by persons involved in loading Bio-Save 10LP Biological Fungicide. Furthermore, precautionary labelling will alert users of the potential sensitization hazard of the end-use products.

The proposed use pattern of Bio-Save 10LP Biological Fungicide as a post harvest treatment for stored produce is expected to result in an increase in residues of *P. syringae* strain ESC-10 on treated produce that is higher than levels naturally occurring on fruit. However, *P. syringae* strain ESC-10 demonstrated no oral toxicity and was not pathogenic/infective via pulmonary or intravenous exposure routes at the maximum dose tested in the Tier I acute toxicity/infectivity studies. *Pseudomonas syringae* strain ESC-10 is also not known to produce any secondary metabolites and there have been no reports of adverse effects to humans from natural populations of *P. syringae*. Therefore, negligible to no risk is expected for the general population, including infants and children, or animals from residues in or on agricultural commodities. Consequently, the establishment of a maximum residue limit (MRL) is not required for *P. syringae* strain ESC-10.

7.2 Environmental Risk

The non-target studies, scientific rationales and published scientific literature submitted in support of *Pseudomonas syringae* Strain ESC-10 Fungicide Technical were determined to be sufficiently complete to permit a decision on registration.

Environmental effects studies and waiver rationales were submitted to address the hazards of *Pseudomonas syringae* strain ESC-10 Fungicide Technical to non-target organisms. These studies, rationales and other published information showed that the use of Bio-Save 10LP Biological Fungicide containing *Pseudomonas syringae* strain ESC-10 Fungicide Technical does not pose a significant risk to birds, mammals, arthropods (including honeybees), fish, non-arthropod invertebrates, plants, or algae.

No additional studies were required to address the environmental fate and behaviour of *Pseudomonas syringae* Strain ESC-10 Fungicide Technical. Environmental fate data (Tier II/III) are not normally required in the absence of significant toxicological effects in non-target organisms in Tier I testing.

As a precaution, standard label statements will prohibit handlers from contaminating aquatic habitats including lakes, streams, ponds or other water bodies.

7.3 Value

The data submitted to register Bio-Save 10LP Biological Fungicide are adequate to demonstrate efficacy for use on apples, pears, cherries and potatoes in suppressing the proposed diseases and pathogens.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Pseudomonas syringae* – strain ESC-10 Fungicide Technical and Bio-save 10LP Biological Fungicide, containing the technical grade active ingredient *Pseudomonas syringae* – strain ESC-10 to prevent fungal rot in stored fruits (apples, cherries and pears) and potatoes.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

°C	degree(s) Celcius
µg	micrograms
ADI	acceptable daily intake
ARD	acute reference dose
bw	body weight
CFU	colony forming unit
EP	end-use product
FAME	fatty acid methyl ester
FDA	Food and Drugs Act
g	gram
g/cc	grams per cubic centimetre
IAA	indole acetic acid
IPM	integrated pest management
kg	kilogram
L	litre
LD_{50}	lethal dose 50%
mg	milligram
mL	millilitre
MPCA	microbial pest control agent
MRL	maximum residue limit
NIOSH	National Institute of Occupational Safety and Health
OEAD	Organization for Economic Co-operation and Development
PCPA	Pest Control Products Act
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
RFLP	restriction fragment length polymorphism
rRNA	ribosomal ribonucleic acid
PMRA	Pest Management Regulatory Agency
TGAI	technical grade of the active ingredient
TSMP	Toxic Substances Management Policy
U.S EPA OPPTS	United States Environmental Protection Agency Office of Prevention, Pesticides and Toxic Substances

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *Pseudomonas syringae* strain ESC-10 and its associated end-use product (Bio-Save 10LP Biological Fungicide)

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Pseudomonas syringae* strain ESC-10				
Acute Oral Toxicity	Rat-Sprague Dawley 15/sex, 2.8×10^7 CFU *P. syringae* strain ESC-10/animal	LD_{50} >2.8×10^7 CFU/animal	-No mortalities or effect on body weight gain and no clinical signs of treatment related toxicity, infectivity or pathogenicity. - No significant findings observed at necropsy. -The MPCA was not reported in the digestive tract of any treated animals by Day 8 except for one female rat displayed the MPCA in the cecum on Day 22. **NON-TOXIC, NOT INFECTIVE** **ACCEPTABLE**	PMRA 1579791
	Rat-Sprague Dawley 5/sex 5010 mg of formulated EP/kg body weight (at 1.46×10^{10}CFU *P. syringae* strain ESC-10/g)	LD_{50} >5010 mg of formulated EP/kg body weight (at 1.46×10^{10}CFU *P. syringae* strain ESC-10/g)	-No mortalities or effect on body weight gain and no clinical signs of treatment related toxicity, infectivity or pathogenicity **NON-TOXIC, NOT INFECTIVE** **ACCEPTABLE**	PMRA 1579787

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Pseudomonas syringae* strain ESC-10				
Acute Pulmonary Toxicity and Infectivity	Rat-Sprague Dawley 15/sex, 8.4×10^8 CFU *P. syringae* strain ESC-10/animal -6 animals per sex for controls.	$LD_{50} > 8.4 \times 10^8$ CFU/animal	-No mortalities or effect on body weight gain and no clinical signs of toxicity. -Microbial counts of the MPCA were reported in the lungs of all treated animals on Day 1 of the study. By Day 4 and at all subsequent sacrifice days, there were no colonies detectable in the lung tissue. -One female rat showed a small number of colonies in the mesenteric lymph nodes on Day 8 of the study. **NON-TOXIC, NON-INFECTIVE** **ACCEPTABLE**	PMRA 1579792

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Pseudomonas syringae* strain ESC-10				
Intravenous Injection Infectivity	Rat-Sprague Dawley 18/sex, approximately. 10^7 CFU *P. syringae* strain ESC-10/animal	LD_{50} >1.0 x 10^7 CFU/animal	-No mortalities, clinical signs of toxicity or gross lesions were observed in any of the test animals -No effect on body-weight gain and no apparent signs of treatment-related toxicity or pathogenicity. -MPCA was reported in the blood of 5 of the 6 treated animals on Day 1 but was no longer detected by Day 4. -Low and sporadic microbial counts were reported in the spleen on Day 4, with clearance achieved after Day 8. **NON-TOXIC, NON INFECTIVE** **ACCEPTABLE**	PMRA 1579799

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Pseudomonas syringae* strain ESC-10				
Acute Dermal Toxicity/ Irritation	Rabbit-New Zealand White, 6 males and 4 females, 5010 mg of formulated EP/kg body weight (at 1.46 x 10^{10}CFU *P. syringae* strain ESC-10/g)	LD_{50} >5010 mg of formulated EP/kg body weight (at 1.46 x 10^{10} CFU *P. syringae* strain ESC-10/g)	-No adverse effects, signs of toxicity were reported. -All test rabbits gained weight on the study. **NON-TOXIC** **ACCEPTABLE**	PMRA 1579800
	Rabbit-New Zealand White, 6 males 500 mg of the formulated EP (at 1.46 x 10^{10}CFU *P. syringae* strain ESC-10/g.		-Very slight grade erythema (Grade 1) was observed in two of six rabbits 30 to 60 minutes and 24 hours after unwrapping. The erythema was resolved by the 48 hour observation, and no other indications of a dermal effect were reported. - After 24 hours the maximum irritation score (MIS) was 0.333/4 with a mean average score (MAS) of 0.111/4. **MINIMALLY IRRITATING** **ACCEPTABLE**	PMRA 1579803

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Pseudomonas syringae* strain ESC-10				
Eye Irritation	Rabbit- New Zealand White, 6 males, 100 mg of 10% end use formulation in one eye of each rabbit		Minimal irritation was reported in all six rabbits at 1 hour and in 4 of 6 rabbits at 24 hours. The irritation was resolved by 48 hours in 5 of 6 rabbits. Irritation was scored by the method of Draize and After 24 hours the maximum irritation score (MIS) was 4.33/110 with a mean average score (MAS) of 1.11/110. Protective eyewear (goggles) is recommended for applicators to reduce risk of contact. **MINIMALLY IRRITATING AND REVERSIBLE** **ACCEPTABLE**	PMRA 1579808

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Organisms				
Vertebrates				
Birds	Oral	A waiver was submitted citing that the use pattern would result in negligible exposure to birds and the inability of the MPCA to grow at temperatures above 32°C. **WAIVER ACCEPTED**		PMRA# 1579811
	Pulmonary	A waiver was submitted citing that the use pattern would result in negligible exposure to birds and the inability of the MPCA to grow at temperatures above 32°C. **WAIVER ACCEPTED**		PMRA# 1579811
Wild Mammals	A waiver request was submitted citing that the use pattern would result in negligible exposure to wild mammals and the inability of the MPCA to grow at temperatures above 32°C. **WAIVER ACCEPTED**			PMRA# 1579811

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Invertebrates				
Arthropods				
Non-target Insect Honeybees	A waiver request was submitted citing that the use pattern would result in negligible exposure to non-target insects and honeybees. **WAIVER ACCEPTED**			PMRA# 1579811
Non-arthropods				
Invertebrates	A waiver request was not submitted. Exposure to non-arthropod invertebrates is expected to be negligible from the indoor use of Bio-Save 10LP Biological Fungicide. **NO FURTHER DATA REQUIRED**			
Plants				
Plants	Cut surface of dormant shoots (sweet cherry and pear)	4 shoots/replicate 3 replicates/group (test groups and controls) 1 replicate of 4 shoots (negative control) Measured concentrations: 1.0×10^8, 1.0×10^7, and 1.0×10^6 CFU/mL Shoots were observed for 13 days	The treatment group showed signs of mild infection in two of the replicates in the form of bacterial ooze in the shoots of sweet cherry. (1.0×10^8 and 1.0×10^7 CFU/mL) **PATHOGENIC**	PMRA# 1579814
Plants	Shoots and leaves (sweet orange, grapefruit, lime, lemon, and avocado)	<u>Shoots</u>: Controls: Four sets of two incisions. Treatments: Four sets of two incisions. Concentration: 1×10^6 CFU/mL <u>Cut Leaves</u>: Controls: Four sets of three leaves. Treatments: Four sets of three leaves. Concentrations: 1×10^6, 1×10^7, or 1×10^8 CFU/mL <u>Punctured Leaves</u>: Controls: 16 punctures/leaf on two leaves. Treatments: 16 punctures/leaf on two leaves. Concentrations: 1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 CFU/mL (measured) <u>Fruit</u>: Controls: 16 punctures/fruit on eight fruit.	Dark brown areas 1 – 2mm in diameter were observed on lime fruit after inoculation with the MPCA. These results indicate that the MPCA can be mildly infective on lime. **PATHOGENIC**	PMRA# 1579813

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
		Treatments: 16 punctures/fruit on eight fruit. Concentrations: 1×10^5, 1×10^6, 1×10^7, and 1×10^8 CFU/mL (measured) Shoots and leaves were observed for 30 days. Fruit was observed for 16 days.		
	Blossom (cherry, pear, prune and apricot); green shoots (cherry, prune, apple, peach and apricot); and trunks (apple and peach)	Green shoots and trunks: Controls: 3 per/replicate; 1 replicate/group Treatment: 3 per/replicate; 1 replicate/group Blossoms: number of blossoms tested varied greatly Concentration: $10^7 - 10^8$ CFU/mL (measured) Duration: Blossom: 7 days Green shoots: Not specified Woody trunks: 18 days	The MPCA may cause blossom blast/blight on cherry and pear cultivars. They may also cause infections on green shoots of Redhaven peach. **PATHOGENIC**	PMRA# 1579812
Aquatic Organisms				
		Vertebrates		
Freshwater fish	A waiver was submitted citing that the use pattern would result in negligible exposure to freshwater fish and an absence of adverse effects reported in the published literature. **WAIVER ACCEPTED**			PMRA# 1579811
Estuarine and marine fish	A waiver was submitted citing that the use pattern would result in negligible exposure to estuarine and marine fish. **WAIVER ACCEPTED**			PMRA# 1579811
		Invertebrates		
Arthropod and non-arthropod invertebrates	A waiver was submitted citing that the use pattern would result in negligible exposure to aquatic invertebrates and an absence of adverse effects reported in the published literature. **WAIVER ACCEPTED**			PMRA# 1579811

Table 3 Alternative Fungicides Registered for Postharvest Diseases on Apple, Pear, Cherry and Potato

Crop	Product	Active Ingredient	Resistance Management Group	Pests
Apple and Pears	Scala SC Fungicide	Pyrimethanil	9	Control of *Botrytis*, Suppression of *Penicillium*
	Mertect SC Fungicide	Thiabendazole	1	Control of *Penicillium* and *Botrytis*
	Scholar 50WP Fungicide	Fludioxonil	12	Control of *Penicillium* and *Botrytis*
Cherry	Scholar 50WP Fungicide	Fludioxonil	12	Control of *Penicillium* and *Botrytis*
Potato	Mertect SC Fungicide	Thiabendazole	1	Control of *Fusarium*

Table 4 Use (label) Claims Proposed by Applicant and Whether Acceptable or Unsupported

Proposed use claim	VSAD comments
For control of decay by blue mould (*Penicillium expansum*), grey mould (*Botrytis cinerea*), and mucor rot (*Mucor piriformis*) on apples and pears, make a single application of 500 g of Bio-Save 10LP per 300 L of water as a post harvest application only. Bio-Save 10LP should be applied prior to disease development. Apply as a conventional dip or drench or as a non-recovery spray to freshly cleaned fruit prior to waxing over soft, clean brushes or donut rolls	Use claim is supported as suppression.
For control of decay by blue mould (*Penicillium expansum*) and grey mould (*Botrytis cinerea*) on cherries, make a single application of 500 g Bio-Save 10LP per 100 L of water as a post harvest application only. Bio-Save 10LP should be applied prior to disease development. Apply as dip or drench or with overhead application system. For overhead application, apply 4L of solution to 900-1800 kg of cherries	Use claim is supported as suppression.
For control of dry rot (*Fusarium sambucinum*) on potatoes, make a single application of 500 g Bio-Save 10LP per ~100 L water as a post harvest application only. Bio-Save 10LP should be applied prior to disease development. Apply over conveyor belt or rollers by drip or spray. Apply entire suspension to 3000 sacks (45.5 kg each) of potatoes.	Use claim is supported as suppression.

References

A. List of Studies/Information Submitted by Registrant

1.0 Chemistry

2.0 Human and Animal Health

3.0 Environment

4.0 Value

B. Additional Information Considered

i) Published Information

1.0 Chemistry

2.0 Human and Animal Health

3.0 Environment

4.0 Value

ii) Unpublished Information

1.0 Chemistry

2.0 Human and Animal Health

3.0 Environment

4.0 Value

Verified Reference List

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